THE PREPARATION OF MATERIAL FOR GENERAL CLASS USE.

THE preparation of material for studies of structure, development, and embryology, for general course students, or for advanced courses where the primary object is to give the student an opportunity to examine as large a series of forms as possible, in order to pave the way for broader generalizations, and yet allow him to do a considerable portion of the work, especially that of the final preparation of the specimens, has been a problem of some little difficulty with me, and which is perhaps shared to some extent by others. One may depend on material simply preserved in alcohol, which the members of the class may section as best they can free hand, but this method does not give such good preparations usually as sections made by some method of precision, though it is a very useful thing to know how to make free hand sections well.

Several laboratories have had recourse to freezing microtomes, or rather to cutting frozen plants with the microtome. This is usually done by the instructor or assistant, and the sections are distributed to members of the class, where the final treatment is given by each individual. This, it has seemed to me, is an excellent method, and while the student does not ordinarily do the sectioning, each one usually has an opportunity to see how the material is oriented, and can in this way gain a good notion of the relation of the section to the part of the plant cut. So well did I think of this method that I was about to introduce it into our laboratories when another method upon which I had been working for about two years seemed to me to be in general a better one, and it has been largely adopted in my general classes. It is understood, of course, that when an individual comes to take up work of the nature of investigation, all the processes involved in the preparation of the material are required to be conducted by him. Usually, also, in the advanced courses which precede investigation, each student is called upon to carry several forms through all the necessary processes of fixing and manipulation, so that there may be some training in methods preliminary to the later work of investigation. It this way persons who later do not take up special lines of investigation will have an opportunity of studying a larger number of forms than would be possible if it were insisted that all the work of preparation should be required, and at the same time there is some

practical knowledge of methods which is especially useful to those who are looking forward to teaching in the secondary schools.

The method is, in brief, to carry the material through all the processes of fixing, dehydration, and infiltration, with some medium in which the sections can be made and have the material ready to section at a moment's notice: not simply to prepare enough material for the use of the class of one year, but to prepare a sufficient quantity at once to meet the wants of a class of ten to twenty students for a period of years. Take for example, among the bryophytes, such liverworts as Riccia, Marchantia, Preissia, Pellia, Pallavacinia, Ptilidium, Cephalozia, etc. To obtain material for classes in several stages of development takes a considerable amount of time. When the material is once found in quantity it requires but little more time to carry through a large amount which will last for a period of years than to prepare just enough for one year. And this is the principle which I have adopted in the preservation of material for class study. The greater amount of material has thus far been prepared by the collodion method, and when once imbedded in collodion the blocks containing the plant parts ready for sectioning are stored in 80 per cent. alcohol, and then are ready to cut on a moment's notice and to serve to the class. For certain material collodion is excellently adapted, while for other material it is poorly adapted, and I have been obliged in many cases to resort to paraffin imbedding, which is far superior for certain kinds of work.

It is unnecessary to give here in detail the processes of fixation, dehydration, and infiltration in collodion. These are sufficiently well known or can be obtained from the books. But it may not be amiss to give briefly the method which I have recently adopted with success in imbedding large quantities of material at one time in collodion. I use collodion made by dissolving ordinary gun cotton in equal parts of 95 per cent. alcohol and ether; two solutions, a thin one of 2 per cent. consistency (2 grams gun cotton to 100^{cc} alcohol and ether), and a thicker one of 5 per cent. consistency.

The objects are previously trimmed to the desired size and form for sectioning. From the vial which holds them the 95 per cent. alcohol is decanted, and if there is considerable bulk of tissue an amount of ether approximately equal to the estimated amount of alcohol remaining in the tissues is added before pouring on the 2 per cent. collodion. This prevents an excess of alcohol which flows out of the

tissues from coagulating a film of collodion on the outer surface which would interfere with infiltration. The objects may remain in the 2 per cent. collodion for twenty-four hours to several days or weeks at pleasure. The 2 per cent. is decanted, and the 5 per cent. poured on, which also may remain for twenty-four hours or more. Care should be used to prevent evaporation in the storage bottles of collodion, or in the vials during infiltration. After replacing the corks the bottles can be inverted for a moment, and the collodion running around the cork seals it. The objects are now poured with the 5 per cent. collodion into shallow paper boxes, the latter being received into vessels ordinarily employed as moist chambers, though there should be no water in the chambers. Here they are allowed to remain for two days or so while the collodion slowly thickens to the desired consistency, when the boxes are immersed in 95 per cent. alcohol for about twenty-four hours. The paper is now stripped from the block of collodion, and the latter is stored in 80 per cent. alcohol.

The paper trays should be lubricated previously on the inside with vaseline so that the paper will easily part from the collodion. The vessel used for a moist chamber should be one which can be partly opened at the top, never at the bottom, for the circulation of air, so that the thickening of the collodion will not be unnecessarily prolonged, and at the same time it must be slow enough to permit all air bubbles, which may be present when the material is poured in the trays, to rise to the upper surface and disappear, and also to permit an even thickening of the collodion lest an outer layer is hardened quickly which prevents the proper hardening of the interior. The trays should be of such depth that they may be filled at once with an amount of collodion which when thickened will be of the desired thickness for sectioning. I usually employ trays from 10 to 15 mm deep. If there is not sufficient 5 per cent. collodion in the vial at the time to fill the tray more is added. The trays vary in size according to the amount of material to be imbedded, and frequently several trays are used for one lot of material. The trays may vary from 5–10 cm long by 3–8 cm in width. As soon as they are filled with the collodion a small needle is employed to adjust the objects in convenient position for orienting, and at such distances that each may be cut out in a block of hardened collodion of such a size as to fit directly in the jaws of the microtome. It thus requires but little time to place the material in the trays in the nearly closed receptacles where evaporation may go

on slowly, and there is no danger that the material will become too hard and dry if it should be overlooked for several hours beyond the usual time required for thickening. Where large trays are needed, I have several times employed Petrie dishes with success.

The material is thus ready for use on the shortest notice, and a sufficient amount for several years. When it is to be used the assistant cuts out an object in a block of collodion of convenient size, places it in the jaws of the microtome properly oriented, sections it, fixes a few sections to the glass slip with ether and alcohol, and the preparation is then handed to the student, or the student may do the sectioning for himself. Stains and after treatment may be used at discretion, and when the preparation is ready for observation and study the student has a permanent one which can be of use afterwards for reference or for demonstrations. I have large quantities of material stored in this way in collodion, some of which has been in this condition for over two years, and the sections this year show that it is in as good condition apparently as when first prepared. In order to show how far the method may be extended with success I will give here a list of the things imbedded in collodion which I have stored in greater or lesser quantity now in the laboratory, usually a sufficient amount to last for from five to ten years, and in some cases for a longer period.

Fungi.—Olpidiopsis saprolegniæ, Synchitrium decipiens, Empusa grylli, Cystopus candidus, Peronospora alsinearum (conidial stage, oogonia, and oospores), P. parasitica (same), P. effusa, Plasmopara obducens, P. halstedii, P. geranii, Ustilago zeæ, Doassansia opaca, D. martinoffiana, Pilacre petersii (from dried material), Crucibulum vulgare, Cyathus striatus, Collybia radicata, Coprinus micaceus, C. atramentarius, Puccinia pimpinellæ (three stages), Puccinia podophylli (two stages), P. asteris, P. orbicula, P. anemones-virginianæ, P. xanthii, P. circææ, P. peckiana (caeoma and spermagonial stage), Uromyces caladii, Phragmidium gracilis (aecidial stage), Phragmidium sp. (aecidial stage), Gymnosporangium macropus, Roestelia on Amelanchier fruit, Melampsora farinosa, Aecidium clematidis, Ae. sambuci, Ae. impatientis, Ae. compositarum, Ae. grossulariae, Ae. podophylli, Magnusiella potentillae, Morchella conica, Discina warneri, Herpotrichia keitii, Xylaria polymorpha, Entomosporium maculatum.

Algæ.—Fucus vesiculosus, Laminaria saccharina, Leathesia difformis, Mesogloea divaricata, Nemalion multifidum, Dasya elegans, Chon-

driopsis tenuissima, Champia parvula, Rhabdonia tenera, Gracillaria multipartita. All the species are in fruit, and the two latter with both tetraspores and cystocarps; cystocarps in the other Florideæ.

Bryophytes.—Antheridia and archegonia and development of the sporogonium in Marchantia polymorpha, Conocephalus conicus, Preissia commutata. Antheridia and archegonia in Pellia endiviaefolia, Pallavicinia lyellii. Development of sporogonium in Aneura sp., Ptilidium ciliare, Cephalozia curvifolia, C. multiflora, Lophocolea heterophylla. Antheridia and archegonia of Mnium affine and cuspidatum.

Ferns.—Sporangia of Pteris albolineata, Aspidium falcatum, Onoclea struthiopteris.

Living material of the ferns is kept in the green houses for complete studies of development, and here the students have practice in methods by carrying the material through all stages of preparation. The same thing is done by them in other groups also. Quantities of other material fixed in various ways are kept at hand in alcohol. Material imbedded in paraffin has not been kept a sufficiently long time to determine the value of this method in the storage of material ready for sectioning, but it may be kept in cedar oil ready for infiltration.— GEO. F. ATKINSON, *Cornell University*.